REMARKS/ARGUMENTS

Compliance with the sequence listing requirements of 37 CFR 1.821(a)(1) and (a)(2).

Applicants request entry of the enclosed substitute sequence listing into the specification in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment contains SEQ ID NOS:1-23 in computer readable form (CRF) and a paper copy of the sequence information which has been printed from the computer readable form. The information contained in the computer readable form was in part prepared through the use of the software program "PatentIn 3.0" and is identical to that of the paper copy.

Applicants have amended the specification in order to correctly identify SEQ ID NOs:5 and 6 as a single polypeptide sequence, (SEQ ID NO:5). Support for this amendment is found, for example, in Figure 5 and at page. 5, lines 23 and 24 of the specification. Applicants have also amended Tables B, C, and D in order to remove the associated SEQ ID NOs. The amino acids listed in the third column of Tables B, C, and D are not meant to be viewed as polypeptide chains, rather they are a list of individual residues that are suitable as substitutions for the corresponding wild type residues identified in the first column of the row. As such, Applicants have further amended the table to add commas between each of the amino acids for clarification purposes. Finally, Applicants have corrected all of the errors cited in the error report sent with the Office Action dated October 22, 2007. These amendments add no new matter.

Accordingly, the Applicants respectfully request entry of the sequence listing and reconsideration and withdrawal of this grounds of objection

Status of the claims

Claims 143 to 146, 148 to 153, and 188 to 193 were previously presented for examination. Claims 143 and 144 are amended herein. Claims 152, 153, and 191 to 193 are herein canceled without prejudice. After entry of these amendments, claims 143 to 146, 148 to 151, and 188 to 190 will be presented for examination on the merits.

Support for the amendments to the claims

Claims 143 and 144 have been amended to remove the recital of specific mutations at residues Y66 and Q69 of the claimed functionally engineered fluorescent protein. These claims have also been amended to list mutations previously set forth as those of Table F and find support accordingly in the specification as filed at p. 36 and 37. These claims have also been amended to set forth "a different anion binding affinity." Support for this recital is found at pp. 36 and 37 which show mutations increasing for small and large anions and, more particularly, at p. 34, lines 14 and 15.

Accordingly, the Applicants believe the amendments to the claims add no new matter and respectfully request their entry.

Response to the rejections for double patenting over claims 1 to 6 and 29 to 37 of U.S. Patent No. 6,150,176 and over claims 1 and 4 of U.S. Patent No. 6,780,975.

Claims 143 to 146, 148 to 153, and 188 to 193 stand rejected under the judicially created doctrine of obviousness-type double patenting over claims 1 to 6 and 29 to 37 of U.S. Patent No. 6,150,176 and over claims 1 and 4 of U.S. Patent No. 6,780,975.

As amended, independent claims 143 and 144 no longer set forth mutations at Y66 and Q69 and thus require *additional* mutations which are *not* set forth in any of the claims of the '176 and '975 patents.

Accordingly, the Applicants respectfully request that the above grounds for rejection be reconsidered and withdrawn.

Response to the rejection of claims 143 to 146, 148 to 153, 188 to 191, and 193 for an alleged lack of enablement under 35 U.S.C. §112.

Claims 143 to 146, 148 to 153, and 188 to 193 stand rejected under 35 U.S.C. §112 for an alleged lack of enablement. Applicants note that claims 152, 153, and 191 to 193 have been canceled without prejudice.

To rebut the rejection and further address specific concerns set forth in the prior Action, the Applicants supplement their earlier *Wands* analysis. We next provide additional

remarks concerning three of the *Wands* factors most at issue: the *Teachings of the Specification*, the *State of the Art*, and the *Amount of Experimentation* required to practice the invention.

Teachings of the Specification:

As evidenced in the enclosed declaration of George Hansen, the specification teaches how to generate a variant of an *Av*GFP-rp which contains up to 63 mutations (73.5% sequence identity), with respect to the wild type protein sequence, and which still retains useful, if not improved, fluorescent activity. This alone is enough for any person skilled in the art, which in this case would include protein design and evolution, to practice the full scope of the invention. Additionally, as discussed in the declaration, the specification teaches how to readily generate and select fluorescent proteins according to the claims, through the incorporation of several references that do exactly this.

The teaching of the high-resolution crystal structure of GFP in the instant specification facilitates the selection of additional mutations that would not significantly alter the fluorescent properties in a variant of an *Av*GFP-rp. The provided structure and description of its functional domains serves to identify regions which may be individually modified to achieve distinct purposes without an expectation of affecting the functioning of other domains. It also identifies individual residues and their environments which further provides a person of ordinary skill in the art with opportunities to conservatively substitute residues with little expectation of greatly altering the activity of a GFP. Applicants respectfully submit, that any structural biologist of average skill in the art, would readily be able to generate a plethora of silent mutations in a variant of an *Av*GFP-rp, through manual inspection of the crystal structure alone.

State of the Art and the Amount of Experimentation Considered Routine:

The state of the art and the amount of experimentation are inter-related. The state of the pertinent experimental methodology is sufficiently advanced that what might otherwise be an enormous amount of experimentation is merely routine. As discussed above, as well as in the declaration submitted by George Hansen, the directed evolution and screening methodologies employed in the field of fluorescent proteins are both routine and rather simple. When these methodologies are coupled with the abundance of biochemical and biophysical knowledge

available for these proteins, as further supported by the teachings of the specification, and the ready availability of many simple mutagenesis and general cloning techniques, also evidenced in the specification, the generation of a vast number of mutations in any fluorescent protein, let alone the mere 36 that are required to practice the full scope of the claimed invention, becomes routine.

Most importantly, a principal premise of the Action is that one of ordinary skill would not be able to create a protein with a diverse combination of mutations. However, whatever may be the general validity of this premise, in this particular art, the premise is wrong. The capabilities in this art are exemplified by the work of Campbell et al. who engineered ***33*** mutations into a GFP-related protein isolated from *Discosoma* coral, dsRed, *without* the aid of vast knowledge, as provided for *Av*GFP in the instant application and these remarks. This clearly shows that the amount of experimentation required to practice the claimed invention is well within the capacity of those in the broader field (Campbell et al., *PNAS* 99:7977-82, 2002, enclosed with IDS, see Abstract of Exhibit I of enclosed declaration). The Campbell et al. mutant had about 85% sequence homology to the wild type dsRed protein and retained useful fluorescent activity, including advantageous shifts in its emission and excitation peaks, with respect to the wild type protein.

As set forth in the attached declaration, the ability of persons of ordinary skill in the art to re-engineer the GFP comes has been demonstrated in fact (*see*, Lawrence et al., *J. Am. Chem. Soc.* 129:10110-10112 (2007), see, Appendix J of the enclosed declaration). Starting from a modified GFP which has about 7 substitutions already from the wild-type GFP, they re-engineered several GFP's having up to an additional 36 amino acids non-conservatively substituted (i.e., negatively charged or neutral amino acids were replaced by an Arg or Lys). In another of the modified GFP proteins, 15 negatively charged or neutral amino acids were replaced by a Glu or Asp. Over all, modified GFPs differing in overall charge from +48 to -30 were made and found to have a GFP fluorescence activity. These results clearly show that the GFP is extreme tolerant to substitutions and highly diverse variants can be readily obtained by persons of ordinary skill in the art.

In view of the extensive disclosure of compatible mutations in the specification, the crystallographic and functional analyses provided therein, the advanced state of the art with respect to the manipulations needed to practice the claimed invention, the relative simplicity with which mutants may be screened in truly enormous numbers, and the *demonstrated* ability of others *in this field of art* to obtain useful fluorescent proteins with less than 85% sequence identities, the Applicants submit that the claimed invention can be practiced with an amount of experimentation which is *not* undue but, rather, routine in the art.

Accordingly, in view of the ample evidence now provided, the Applicants respectfully request that the above grounds for rejection be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,

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